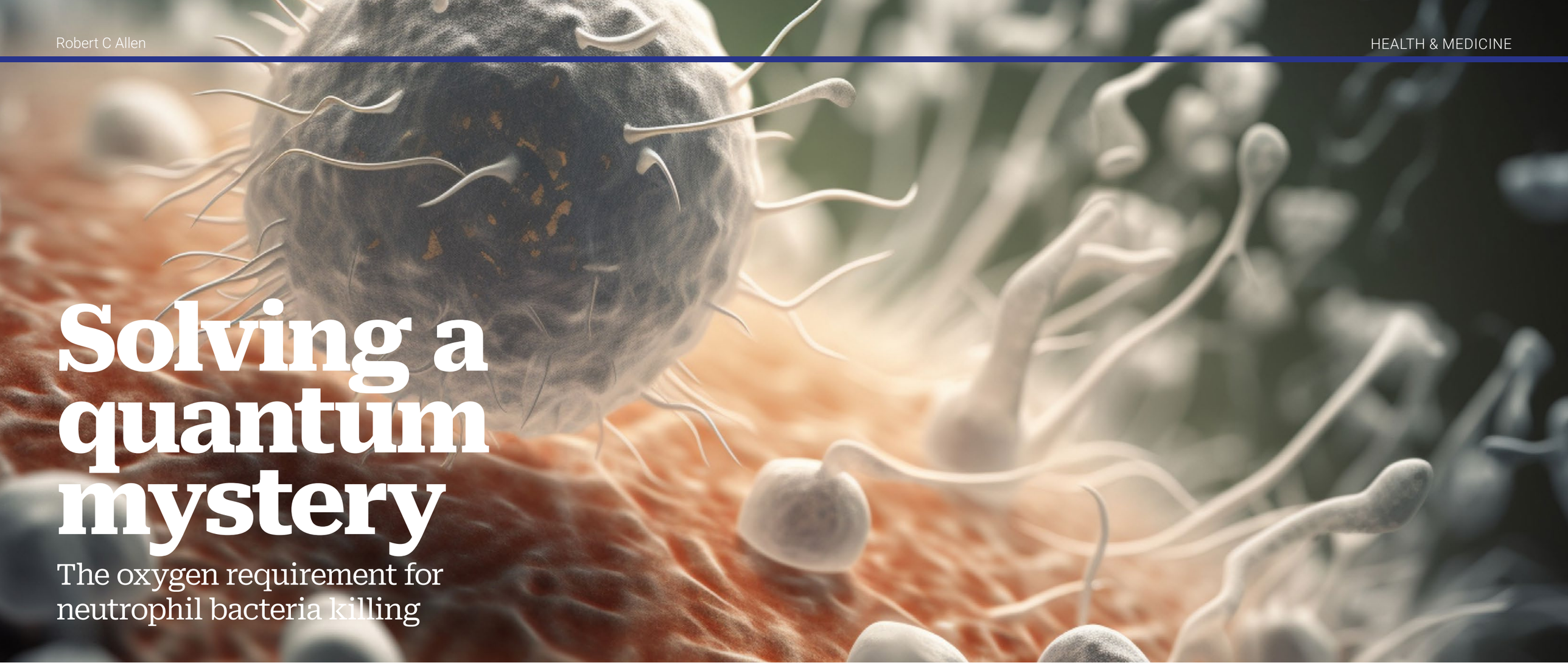


Solving a quantum mystery

The oxygen requirement for neutrophil bacteria killing

- Oxygen plays a crucial role in the immune system, acting as a powerful bactericide in white blood cells. The mechanism explaining this anti-pathogen activity is, however, poorly understood.
- Robert C Allen shows that the conversion of oxygen to the singlet multiplicity quantum state is the key. Extremely potent but short-lived singlet oxygen generated by neutrophils effectively kills bacteria.
- The microsecond lifetime of singlet oxygen requires proximity for effective reactivity but has the advantage of focused reactivity with minimal collateral damage.
- The oxygenation activity of singlet oxygen yields electronically excited carbonyl functions that emit light, providing a powerful way to study its antibacterial activity in real time.

Oxygen plays a crucial role in sustaining life on Earth, supporting the existence of a multitude of organisms. It is an indispensable component of cellular respiration and drives the metabolic processes that enable living beings to extract energy from nutrients. Aerobic organisms, from the tiniest microorganisms to complex multicellular life forms, depend on oxygen for the efficient utilisation of the metabolic reducing equivalents that drive cell function.

Oxygen and the immune system

Oxygen is also a critical element in the immune system. In the human body, neutrophils, a type of white blood cell, are responsible for orchestrating a frontline defence against microbial invaders. These cells realise the reactive potential of oxygen by changing oxygen's spin multiplicity through a process collectively referred to as the respiratory burst. This altered spin state of oxygen is antimicrobial, and the mobile nature of the neutrophils makes them available in any area of the body affected by pathogens through the blood flow.

A peculiar molecule

Although the role of oxygen in supporting a healthy immune system has long been recognised, the details of how oxygen interacts with the complex biochemical machinery of the immune system remain poorly appreciated. The chemistry and biochemistry of oxygen exhibit very peculiar features, setting it apart from other common molecules that interact with our bodies. Professor Allen has devoted more than 50 years to exploring what makes oxygen so special for the immune system, and why this molecule could not be replaced by any other. Drawing from an understanding of oxygen's chemistry, Allen provides an elegant model as to how neutrophils fight microbes. The key to understanding why oxygen is so important for life and its sustenance, says Allen, relates to its frontier orbital electrons responsible for reactivity and is best considered at the quantum mechanical level.

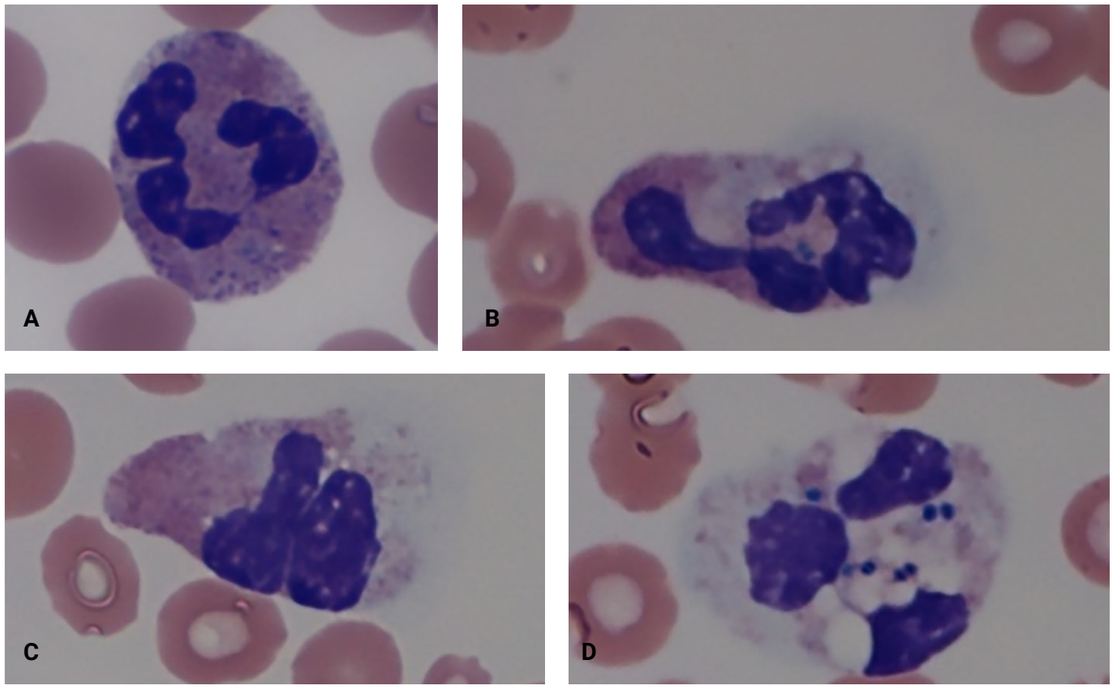
Chemical bonds

Molecules are groups of atoms that exist as individual stable species because of the presence of chemical bonds between those atoms. When molecules interact with each other, for instance during chemical reactions, bonds are broken and new bonds formed, to give rise to new molecular species. Chemical bonds are established when two atoms share one electron each, and these electrons pair close to each other in the region between the two atoms – providing the glue that keeps a molecule together. Sometimes atoms contribute more than one electron to create bonds, which leads to the formation of multiple bonds. For instance, within each molecule of nitrogen (N_2), which is the major constituent of the atmosphere, three chemical bonds are established between the two N atoms. Oxygen itself (O_2) contains a double bond. There is, however, a very important difference in the way electrons are arranged in the bonds of O_2 compared to those of other molecules, like N_2. This is what makes oxygen a unique chemical species.

Direct oxygenation activity by neutrophils requires spin conservation, and the light emission from such bactericidal oxygenations confirms generation and participation of singlet multiplicity oxygen.

The quantum mechanics of oxygen

Electrons are quantum particles that possess a fundamental physical property, or quantum number, known as spin. The spin of an electron can only assume two values, which are usually labelled 'spin-up' and 'spin-down'. When two electrons pair to form a chemical bond, they must have different spins. This is what happens, for instance, in N_2;

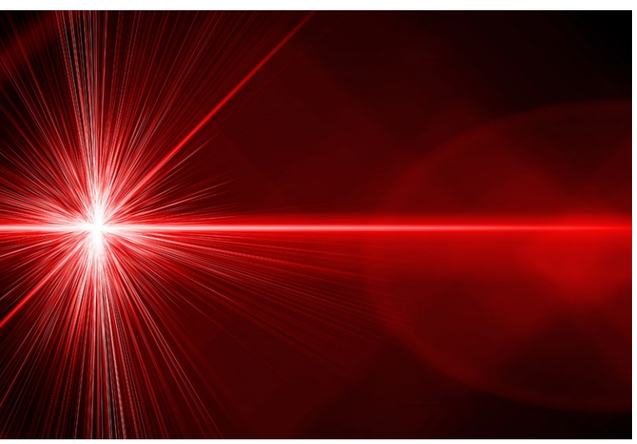


A: Unstimulated neutrophil. B: Cytokine-stimulated neutrophil showing polar-shape change and partial degranulation following phagocytosis of a diplococcal bacterium. C: Neutrophil with partial degranulation, no phagocytosis. D: Neutrophil containing phagocytosed bacteria.

each one of the three bonds of this molecule contains exactly one spin-up and one spin-down electron. Oxygen, however, is different. In the O_2 molecule, two electrons with the same spin value occupy two equivalent energy levels, rather than pairing with opposite spins. The O_2 molecule is therefore a di-radical, that is, a species with two unpaired electrons. In quantum mechanics terms, this peculiar electronic state is called a triplet.

How oxygen reacts

Whereas O_2 has unpaired electrons, many of the molecules with which it interacts on Earth, including most of the organic molecules that constitute living organisms and materials derived from them, possess a singlet state, with no unpaired electrons. Chemically, singlets and triplets do not successfully react with each other. This explains why flammable materials, such as wood or fuels, do not burn spontaneously when in contact with the atmosphere, which contains about 20% of triplet oxygen. However, supplying a modest amount of energy, for instance by ignition with a lighter or a match, is sufficient



to convert the singlet flammable material to two paramagnetic doublet molecules capable of reacting with triplet O_2 and self-sustaining chemical oxidation, or combustion. During combustion, energy is released in the form of heat and light. According to Allen, the generation of singlet oxygen allows direct reaction with singlet biological molecules and is the most important step in the bactericidal action of neutrophils.

Chemiluminescence

Allen has studied how bacteria trigger the response of the neutrophils in the body, by inducing the controlled formation of singlet oxygen. This process is catalysed by enzymes such as NADPH oxidase, and plays an important role in the respiratory burst by promoting the oxidation reactions that kill the bacteria. Singlet oxygen is extremely potent but short-lived. The respiratory burst metabolism of neutrophils drives production of singlet oxygen and microbe killing. These reactions, like those involved in the combustion of burning, emit energy in the form of light. Allen reasoned that light emission, or chemiluminescence, would follow if spin was conserved. Spin conservation is a fundamental law of quantum mechanics. Chemiluminescence provides information with regard to the nature of reaction, and measuring such luminescence provides a wealth of information on the intensity of the oxygenation reactions that occur in vivo. Such measurements and the additional application of chemiluminigenic probes have allowed for the development of fast and non-invasive diagnostic tools to monitor in real time how the body responds to microbial attack or to potentially stressful environments.

According to Allen, neutrophil conversion of O_2 multiplicity from triplet to singlet removes the spin conservation barrier restricting the direct oxygenation reactions responsible for bactericidal action and light emission.

Personal response

What prompted you to study how oxygen acts as an anti-pathogen agent in the body?

My journey in science has been relatively unique. In high school, college, and later in military service my interests were directed to the interrelatedness of systems and unusual phenomena. I was attracted to philosophy and epistemology, especially the work of Kant. My abiding interest in oxygen and combustive action began in high school. None of my teachers were able to adequately answer my questions as to why combustion is not spontaneous. After graduation from college, I began two years of military service starting in the infantry but having a college degree in biology-chemistry, was later assigned to technical work in clinical laboratory medicine. It was in that capacity that the ubiquitous polymorphonuclear leukocyte (typically referred to today as the neutrophil leukocyte [or neutrophil]) captured my attention.

On completion of military service in 1970, and with the encouragement of Randolph M Howes, a schoolmate in college, I began graduate work in biochemistry at Tulane University School of Medicine in my hometown of New Orleans, Louisiana. I was especially interested in the research of Richard H Steele who had spent several years as a post-doctoral fellow with Albert Szent-Györgyi. I had enormous respect for the research and writings of Szent-Györgyi. Professor Steele accepted me as a student and I began working on several projects related to riboflavin redox action and microsomal mixed function oxidase action. My interests in quantum chemistry and spectroscopy were rekindled and I began in-depth studies into these areas, especially the works of Gerhard Herzberg and Paul Dirac.

One of the central findings of your work is that oxygen-driven processes in neutrophils have important analogies with combustion. What are the main similarities and differences between the two?

In the fall of 1971, over routine afternoon coffee with Professor Steele, I divulged my conceptualised understanding as to how neutrophil leukocytes kill microorganisms by a type of inverse combustive action in which ground state triplet molecular oxygen ($^3O_2$; the numeric superscript 3 indicates multiplicity) is univalently reduced by NADH or NADPH flavoprotein oxidase resulting in doublet multiplicity hydroperoxyl acid radical ($^2HO_2$) with acid dissociation yielding its conjugate base doublet multiplicity superoxide anion ($^2O_2^-$). The subsequent disproportionation of this radical pair by doublet-doublet annihilation would yield singlet multiplicity hydrogen peroxide ($^1H_2O_2$) and metastable electronically excited singlet molecular oxygen ($^1O_2$*). Generation of $^1O_2$* removes the reactive barrier imposed by Wigner spin conservation and allows the exergonic reactive potential of oxygen to be realised. The $^1H_2O_2$ produced serves as substrate for myeloperoxidase (MPO) oxidation of singlet multiplicity chloride ($^1Cl^-$) to singlet multiplicity hypochlorite ($^1OCl^-$). Direct reaction of hypochlorite with an additional $^1H_2O_2$ yields $^1Cl^-$ and $^1O_2$*. As such, the neutrophil leukocyte has two possible pathways to $^1O_2$* generation.

The generation of light (photon emission or chemiluminescence) by neutrophil leukocytes engaged in microbe killing would be expected if respiratory burst metabolism (ie, increased $^3O_2$ consumption and hexose monophosphate shunt metabolism) were directed to the generation of $^1O_2$* as described. Light is the consequence of $^1O_2$* reactions with singlet bio-organic molecules resulting is exergonic dioxygenation reactions sufficient for electronic excitation. Production of endoperoxides and dioxetanes by such $^1O_2$*-driven dioxygenations would yield electronically excited singlet multiplicity carbonyl functions that relax by emitting blue photons.

Why is chemiluminescence important and how can it be used to study the complex biochemical processes involved in the body's fight against external pathogens?

$^1O_2$* is in a metastable electronically excited state that has a finite microsecond reactive lifetime. If it does not react, it can relax by emitting an infrared photon. When generated in abundance, two $^1O_2$* molecules can simultaneously relax by emitting a red photon with the combined energy of the two infrared photons. Arguments for such a possibility were made, but not by me. Such emission would represent loss of $^1O_2$* microbicidal potential. In addition, the photomultiplier tubes used for light measurements where significantly less sensitive in the red range of the spectrum. My thinking was as described in the schematic of Figure 4 from Allen et al (1972) *Biochem Biophys Res Comm*. This publication is also the first to correctly predict the generation of superoxide anion and correctly assigned its generation to the flavoprotein NADPH oxidase. In essence, the detection of light in association with neutrophil microbicidal action serves as proof for $^1O_2$* generation by neutrophil leukocytes. Likewise, the haloperoxidase action of purified MPO also results in microbicidal action and light emission. Under conditions where H_2O_2 is limiting, MPO can catalyse chlorination of bio-organic substrates, but such reactions are insufficiently exergonic for electronic excitation and no light is emitted. The acid optima generation of light by MPO haloperoxidase action is consistent with $^1O_2$* generation and resulting dioxygenation activity.

Details

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Bio

Professor Robert C Allen's published research started in 1972, with the report that neutrophils engaged in microbe killing emit light in the visible range of the spectrum. In a seminal paper in 1986, while serving as a military medical officer at the US Army Institute of Surgical Research (aka Burn Center), FSH TX, he demonstrated that neutrophil oxidase and haloperoxidase dependent oxygenation activities could be differentially quantified in real time and with high sensitivity using chemiluminigenic probes (ie, luminol and lucigenin). In recent decades, Allen has applied luminescence measurement techniques and discriminant statistical analysis to evaluation of host systemic inflammation and diagnosis of infectious states. His work with Exoxemis Inc began in 1987 and has focused on improving blood neutrophil luminescence analysis, and on haloperoxidase microbe killing, especially regarding myeloperoxidase binding selectivity resulting in selective killing. More recently, endotoxin inhibition by direct haloperoxidase binding in the absence of haloperoxidase action has also been reported.

Further reading

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